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Resistance of peas to *Sclerotinia sclerotiorum* in the *Pisum* core collection

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In this study, 497 pea accessions from the *Pisum* core collection located at the USDA-ARS, Western Regional Plant Introduction Station (WRPIS), Pullman, WA and seven woody-stem pea lines from a private collection in the UK, were screened for resistance to *Sclerotinia sclerotiorum*, the cause of white mould. All of the *Pisum* genotypes screened were susceptible to infection, and 237 of the 504 genotypes were highly susceptible since these did not survive 2 weeks post-inoculation. However, 22 pea accessions and one woody-stem line were identified with quantitative partial resistance to white mould. Pea accessions 103709, 166084, 169603, 240515 and 270536 from the core collection demonstrated the greatest quantitative partial resistance to *S. sclerotiorum* based on nodal resistance and plant survival in replicated greenhouse and laboratory tests. Only five of the 504 genotypes screened had a mean lesion length of between 0 and 1 cm when assessed 3 days post-inoculation. Pea stem diameter was significantly ($P \leq 0{\cdot}03$) negatively correlated with stem lesion length in replicated greenhouse and laboratory experiments, and was determined to be the best predictor of quantitative partial resistance to *S. sclerotiorum* based on lesion length.

Keywords: disease resistance screening, *Pisum sativum*, *Sclerotinia sclerotiorum*, white mould

Introduction

White mould, caused by the ascomycete *Sclerotinia sclerotiorum*, can be a major foliar disease of both irrigated and dry land peas in the Pacific Northwest and Midwest regions of the USA (Hampton & Ford, 1965; Fenwick, 1969; Muehlbauer *et al*., 1983) and in pea production areas worldwide (Kraft & Pfleger, 2001). The disease is favoured by extended periods of cloudy, cool, wet and humid conditions following row closure, especially when vine growth is heavy, overhead sprinkler irrigation is used, or rainfall is excessive. The pathogen is capable of infecting all above ground foliage and lesions are characterized by a white cotton-like mass of mycelium growing on the surface of stem, leaf or pod tissue. Infected tissue becomes soft and slimy with a water-soaked appearance, but upon drying appears bleached in colour when compared to normal senescent tissue.

White mould of peas is currently managed through an integrated approach which includes: use of fungicides applied during flowering; planting disease-free seed; using three to five year rotations with non-host crops; deep ploughing of sclerotia in infested soils; and promoting open-vine habits to limit humidity within the canopy (Kraft *et al*., 1996; Agrawal & Prasad, 1997; Kraft & Pfleger, 2001). Several of these measures such as long-term rotation and deep ploughing are not practical in many growing areas, and due to poor economic returns, foliar fungicides are cost prohibiting for many pea growers. Since cultural and chemical management tools are either less effective or expensive, the development of resistant pea cultivars to effectively manage white mould is a potentially advantageous but currently unavailable management practice.

Limited screening has been conducted to identify genetic resistance to white mould in peas. However, nine PI accessions from the USDA Plant Introduction Station at Geneva NY, two lines from an Idaho, USA pea collection, and the commercial cultivars Dark Skin Perfection, Perfection 132 and Wisconsin Perfection, have been identified as being more resistant to *S. sclerotiorum* than the susceptible commercial pea cultivar Garfield when screened using a colonized oat inoculation technique (Blanchette & Auld, 1978).

The objective of this study was to identify novel sources of quantitative partial resistance to *S. sclerotiorum* in *Pisum* germplasm collections that would be beneficial to pea breeders in developing cultivars with resistance to this pathogen.

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Table 1 Number of species, subspecies and varieties in the *Pisum* core collection evaluated for quantitative partial resistance to *Sclerotinia sclerotiorum* and the number and percentage of resistant genotypes per taxon

Taxon	No. of genotypes tested	No. of genotypes with partial resistance[a]
Pisum sativum	421	18
Pisum sativum (woody-stem)	7	0
P. sativum subsp. *abyssinicum*	13	0
P. sativum subsp. *asiaticum*	3	0
P. sativum subsp. *elatius*	31	2
P. sativum subsp. *sativum*	16	1
P. sativum *var.* arvense	9	0
P. sativum var. *pumilio*	4	1
Total	504	22

[a]Number of pea genotypes identified with quantitative partial resistance to *S. sclerotiorum* within a given taxon. Genotypes were considered to demonstrate quantitative partial resistance if they had a nodal resistance value greater than 1 and a 50% plant survival rate.

Materials and methods

Seed source

Pea seed representing 497 accessions from the *Pisum* core collection were obtained from the USDA-ARS, Western Regional Plant Introduction Station (WRPIS) in Pullman, WA, and seven pea lines developed by a private pea breeding company from Cambridge, UK, referred to as ICI lines. The core collection contains the *Pisum* accessions that represent the greatest genetic diversity within the *Pisum* collection at the WRPIS (Coyne *et al*., 2005). The accessions originate from 63 countries and consist of seven species, subspecies or varieties of *Pisum sativum* (Table 1).

Plant preparations

Four pea seed of each genotype were planted at a depth of 1 cm in pasteurized soil contained in a 10 cm diameter clay pot. The soil consisted of a mixture of 85 L of Special Blend Soil Mix (Sun Gro Horticulture), 113 L of propagation grade-course perlite (Supreme Perlite Company) and 900 g of Scotts Osmocote Classic 14-14-14 (The Scotts Company). Plants were grown in a greenhouse under natural sunlight and temperatures ranged from 15 to 25°C. Supplemental lighting from 1000-watt halogen lights was used as needed to maintain a photoperiod of approximately 14 h/day.

Inoculum and greenhouse experiments

Sclerotinia sclerotiorum isolate Scl02, from our collection, isolated in 2003 in Quincy, WA and pathogenic to pea, was used for inoculations. Sclerotia of the isolate that were stored at –20°C were transferred to Petri dishes containing potato dextrose agar (PDA) (Difco) and were incubated in the dark at 25°C until the fungus colonized the agar. A Unispense Microprocessor Controlled Dispenser (Wheaton Science Products) was used to dispense 14 mL of agar into each dish, to standardize the size of agar plugs used to inoculate the plants. Agar plugs containing fungal mycelia were then removed from the leading edge of the expanding colony and transferred again to PDA and incubated in the dark at 25°C for 2 days. Mini-agar plugs colonized with mycelia were then taken from the leading edge of the expanding colony using an amalgam carrier (Pulpdent.com). The mini-agar plugs measuring 3·2 mm in diameter by 7 mm in length (56·3 mm^3) were applied to the 4th node of each 15-day-old plant at the attachment point where the leaf branches from the main stem. The 4th node is described as the node in the 4th position when counting the trifid bracts as the first node and numbering successive nodes towards the top of the pea plant.

Following inoculation, the plants were transferred to a humidity chamber in the greenhouse at 100% RH and temperatures ranging from 15 to 28°C and covered with a nylon mesh shade cloth that maintained sunlight between 0·0 and 10 w m^{-2} as measured by a Silicon Pyranometer Sensor (Spectrum Technologies). The purpose of the shade cloth was to create low light intensities that would simulate cloud cover or dense canopy cover, since white mould development is favoured by prolonged periods of cloud cover and tends to develop following row closure (Kraft & Pfleger, 2001). The plants were maintained in the chamber for 3 days at which time the white mould lesions were measured. The plants were then placed on a greenhouse bench with temperatures between 15 and 25°C and a photoperiod of 14 h/day and maintained for an additional 2 weeks, at which point they were assessed for survival and nodal resistance.

Survival was based on whether the plants were still living 2 weeks post-inoculation. Nodal resistance was based on the movement of the lesion down the stem of plants that survived 2 weeks post-inoculation. The following severity values were assigned for nodal resistance: 0 = plant did not survive, 1 = lesion expanded down the stem from the 4th inoculated node to the 1st node, 2 = lesion expanded from the 4th to the 2nd node, 3 = lesion expanded from the 4th to the 3rd node, and 4 = lesions did not expand beyond the initial inoculation point at the 4th node. Half values were also assigned. For example, lesions that were between nodes 3 and 4 were given a value of 3·5.

Three pots each of the white-mould-susceptible pea cv. Bolero containing four plants per pot were used as inoculated and non-inoculated controls for each screening session. A mini-agar plug that did not contain *S. sclerotiorum* mycelia was applied to the fourth node of the non-inoculated plants. A total of 11 primary screening trials with a mean of 53 genotypes screened per trial were performed.

Eighty-eight of the initial 504 genotypes demonstrated white mould resistance based on one or more of the

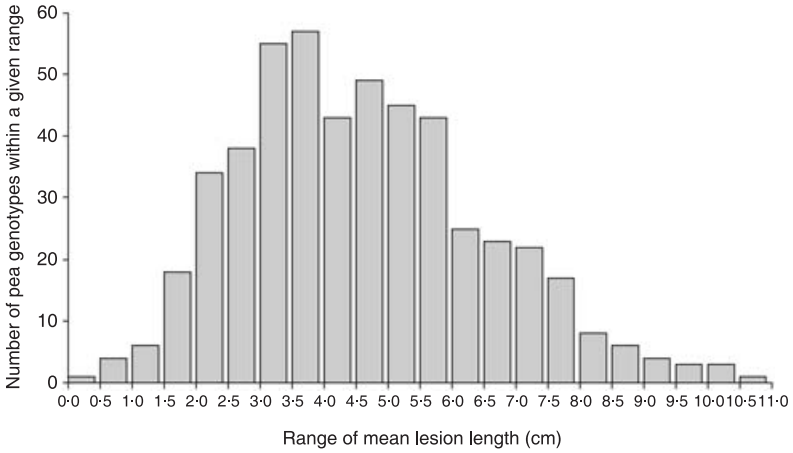

Figure 1 Number of pea genotypes with mean stem lesion lengths ranging from 0–0·5 cm to 10·5–11 cm, when lines were inoculated with *Sclerotinia sclerotiorum* at the fourth node.

following three factors: (i) mean lesion size was 2·5 cm or less, (ii) mean nodal resistance was greater than or equal to 1, and (iii) plant survival was 25% or greater. These were further assessed in separate replicated trials under greenhouse and laboratory conditions. All 88 genotypes were screened at the same time for each trial to standardize the environmental screening conditions. Greenhouse conditions and experimental designs were the same as previously described. Genotypes screened under laboratory conditions followed the same experimental design as in the greenhouse, but were maintained at 10°C for 3 days in a humidity chamber in the dark at 100% RH to provide an extreme screening condition to assess white mould resistance. An evening temperature of 10°C is common in pea production areas in the Pacific Northwest during pea row closure, which favours white mould development. Plants were inoculated and lesion length, nodal resistance and survival data were assessed as previously described.

Data analysis

Since stem diameter was previously noted as being potentially correlated with white mould resistance (Blanchette & Auld, 1978), data regarding the stem diameter of 62 PI accessions, presently screened for white mould resistance, were obtained from a previously published study (McPhee & Muehlbauer, 1999). In addition to information on stem diameter, data regarding internode length, stem shearing strength and stem crushing strength were also obtained for these 62 PI accessions (McPhee & Muehlbauer, 1999), based on the theory that these factors may be related to quantitative partial resistance to *S. sclerotiorum*. For example, stem shearing strength and crushing strength could correlate with stem lignin levels or concentrations of other stem hardening compounds that may correspond to quantitative partial resistance to *S. sclerotiorum*. Correlations among stem diameter, internode length, stem shear strength and stem crushing strength versus lesion length, nodal resistance and survival were assessed using Pearson correlation coefficients calculated by a PROC CORR procedure in SAS.

Greenhouse and laboratory trials were arranged in a completely randomized design. Each plant per pot was considered a replication (four plants per pot). Means for lesion lengths and nodal resistance were calculated using an ANOVA, PROC GLM procedure in SAS (SAS Institute Inc.). To assess whether genotypes evaluated in independent experiments resulted in the same ranking of genotypes between the two experiments for nodal resistance and lesion expansion, a non-parametric analysis of variance based on ranks was conducted using a PROC RANK followed by a PROC ANOVA procedure (Grünwald *et al*., 2003).

Results

Primary white mould screening results

Of the 505 (total includes the cv. Bolero control) pea genotypes screened in the 11 primary screening trials, 5, 26, 77, 109, 91 and 197 genotypes had lesion lengths of 0–1, 1·1–2·0, 2·1–3·0, 3·1–4, 4·1–5, and > 5 cm, respectively (Fig. 1). Mean nodal resistance of the 504 genotypes ranged from 0 to 3·88 with 53% having a rank of zero, indicating that 237 of the 504 genotypes screened did not survive 2 weeks post-inoculation (Fig. 2). All of the *Pisum* genotypes screened were susceptible to infection but varied in response to lesion expansion. Six of seven woody-stem ICI-lines did not survive 2 weeks post-inoculation. However, ICI-line 1204-3 had a mean lesion length of less than 1 cm (0·8 ± 0·5 cm) which was recorded for only five of the 505 genotypes screened (Fig. 1).

Eighty-eight of the initial 504 genotypes screened demonstrated white mould resistance based on at least one of the following three factors: (i) mean lesion size was 2·5 cm or less, (ii) mean nodal resistance was greater than or equal to 1, and (iii) survival was 25% or greater (Table 2 lists the 88 genotypes selected). Therefore, 413 pea PI accessions were considered to be highly susceptible to *S. sclerotiorum* based on the greenhouse screening technique.

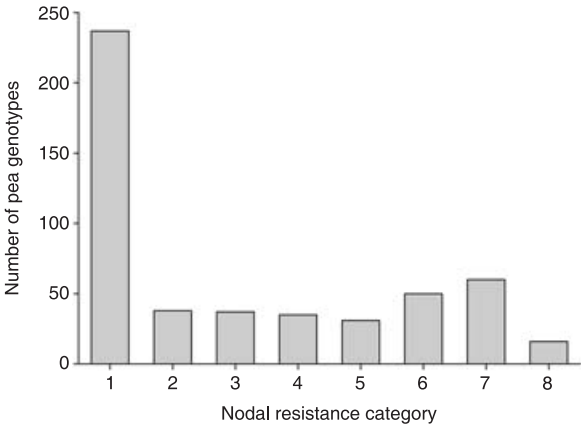

Figure 2 Nodal resistance category of 504 pea genotypes screened for resistance to *Sclerotinia sclerotiorum* based on primary screening results. Category 1 = highly susceptible and 8 = most resistant (see Materials and methods for details).

Laboratory and greenhouse re-screening of potential white mould resistant genotypes based on primary screenings

Twenty-two of the 88 genotypes that were re-screened based on primary screening results, demonstrated a mean nodal resistance of 1 or greater, and survival of 50% or greater, in at least one or more of the replicated trials under laboratory and greenhouse conditions (See genotype numbers with asterisks in Table 2). A mean nodal resistance of 1 or greater and survival of 50% or greater was demonstrated in one or both trials by 15 of the 88 genotypes under laboratory conditions, and 12 genotypes under greenhouse conditions (Table 2). Only five accessions (PI accessions 103709, 166084, 169603, 240515 and 270536) demonstrated this same level of resistance in at least one or more trials under both laboratory and greenhouse conditions (Table 2). Only three genotypes, 244121, 411141 and 1204-3 had lesion lengths of 2·5 cm or less in replicated greenhouse and laboratory trials but none of these genotypes survived 2 weeks post inoculation (Table 2). Line 1204–3 was the only line that in replicated greenhouse trials showed a mean lesion length that was significantly less ($P < 0{\cdot}05$) than that of the Bolero susceptible control. Only 4, 7, 6 and 4 genotypes of the top 30 ranked genotypes for shortest lesion length survived in trials 1 and 2 of both greenhouse and laboratory screenings, respectively.

Plant traits that predict white mould resistance

The Pearson correlation coefficients for stem diameter and lesion length were significantly negatively correlated in replicated laboratory (–0·517 ($P < 0{\cdot}0001$) and –0·352 ($P = 0{\cdot}005$)) and greenhouse (–0·376 ($P = 0{\cdot}003$) and –0·276 ($P = 0{\cdot}03$)) screenings, indicating that as stem diameter increased, lesion length decreased (Table 3). The Pearson correlation coefficients for internode length and lesion length were significantly positively correlated in two of four trials, 0·236 ($P = 0{\cdot}064$) and 0·281 ($P = 0{\cdot}027$), indicating that as internode length decreased, lesion length decreased (Table 3).

Although not statistically significant ($P > 0{\cdot}05$), in all four trials the correlation coefficients for stem crushing strength and lesion length were negatively correlated (–0·151, –0·074, –0·218 and –0·032) and in one of four trials, the correlation coefficient for stem shearing strength and lesion length was significantly negatively correlated with lesion length, –0·311 ($P = 0{\cdot}015$). There were no significant correlations between nodal resistance or survival, and internode length, stem diameter, stem crushing and shearing strengths, except in one of four trials where survival and stem diameter were significantly negatively correlated, –0·271 ($P = 0{\cdot}035$). For both stem crushing and shearing strengths, and lesion length and nodal resistance were always negative correlations (Table 3).

Data analysis

The non-parametric test used to assess the repeatability of the inoculation method in consistently ranking the pea genotypes for white mould resistance between experiments determined that nodal resistance rankings between replicated laboratory and greenhouse experiments were not significantly different for 71 and 74 of the 89 genotypes screened, respectively, and lesion expansion rankings were not significantly different for 62 and 69 of the 89 genotypes screened, respectively. Many of the pea accessions from the *Pisum* core collection are mixed populations of different genotypes from a given collection site, therefore not all the accessions are pure, and from one test to the next there can be genetic variability among the peas selected from a given accession that can contribute to variation in the rankings from one experiment to the next.

Discussion

The purpose of this research was to rapidly screen pea genotypes from the *Pisum* core collection and private breeding lines for quantitative partial resistance to *S. sclerotiorum*. Twenty-two genotypes were identified with quantitative partial resistance. These genotypes originated from 14 countries and consisted of *Pisum sativum*, *P. sativum* subsp. *elatius*, *P. sativum* subsp. *sativum* and *P. sativum* var. *pumilo*, indicating a potential high level of genetic diversity for white mould resistance associated with these genotypes based on the wide origin of accessions and sub-species/variety diversity (Table 4). The most common origin of these white mould resistant accessions were India and Turkey, which were each represented by five accessions. PI accessions 103709, 166084, 169603, 240515 and 270536 from the *Pisum* core collection demonstrated the greatest quantitative partial resistance to *S. sclerotiorum* based on nodal resistance and survival in replicated greenhouse and laboratory tests (Table 2).

Table 2 Mean lesion length, lesion rank, nodal resistance, nodal rank and percentage survival of selected pea genotypes when plants were inoculated with *Sclerotinia sclerotiorum* and maintained in a humidity chamber for 3 days in a greenhouse at 15 to 28°C (Trials 1 and 2) or maintained at 10°C in the dark (Trials 3 and 4)

Genotype[a]	Lesion length[b]	Lesion rank[c]	Nodal resistance[d]	Nodal rank[e]	Percentage survival[f]
Bolero	27·7/28·5/**33·0/25·9**[g]	19/24/21/10	0·0/0·33/0·0/0·02	9/10/14/6	0/33/0/8
103709***	32·0/17·8/35·5/37·8	26/9/30/29	**0·0/2·00/1·50/1·13**	9/2/6/2	0/100/50/75
140298	53·8/38·5/65·8/58·8	56/46/67/63	0·0/0·0/**0·5/0·88**	9/11/12/4	0/0/25/50
141966	51·7/53·3/54·5/47·3	51/66/54/43	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
156720*	**53·0/19·5**/41·8/39·0	55/13/37/31	**0·0/1·5**/0·5/0·0	9/4/12/7	0/75/25/0
164971	44·0/35·3/52·3/50·3	42/38/51/49	**0·5/0·0/0·0/0·88**	7/11/14/4	50/0/0/50
164972**	54·0/39·8/**49·3/33·0**	57/49/46/24	0·0/0·0/1·75/1·5	9/11/3/3	0/0/75/67
165949*	50·5/43·3/61·8/50·3	50/56/62/50	0·0/0·0/**1·63/0·0**	9/11/5/7	0/0/75/0
166084**	55·5/42·8/56·0/38·7	60/55/56/30	**0·0/1·25/0·5/2·67**	9/5/12/1	0/50/25/100
169603**	**29·8/36·8**/34·8/23·3	21/42/28/5	**2·0/0·0/1·75/0·0**	3/11/3/7	75/0/75/0
171810*	**54·3/36·8/41·0/24·3**	58/42/36/7	0·0/0·0/**0·5/1·17**	9/11/12/3	0/0/33/67
174320	27·5/32·3/43·8/48·3	18/33/39/45	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
180696	37·8/29·0/**40·0/53·3**	35/27/33/53	0·0/0·0/0·5/0·0	9/11/12/7	0/0/25/0
181801	48·0/35·8/34·0/29·5	47/39/25/17	0·0/0·0/0·63/0·0	9/11/11/7	0/0/25/0
184131	**32·0**/**14·5**/31·0/26·3	26/617/12	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
195404	46·3/31·8/**33·5/47·5**	44/31/24/44	0·0/0·75/0·0/0·0	9/8/14/7	0/25/0/0
197044**	**47·8/24·8**/40·7/46·5	46/20/35/41	0·0/0·63/1·67/1·38	9/9/4/2	0/25/67/75
204306	**33·0/44·3/66·0/27·5**	27/57/68/14	0·0/0·0/**0·0/0·63**	9/11/14/4	0/0/0/50
210561	81·8/49·8/67·3/65·5	70/62/69/69	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
227258*	**55·8/28·8**/54·3/48·8	61/26/53/47	**0·0/1·63**/0·0/0·0	9/3/14/7	0/75/0/0
240515**	**48·8/24·8/42·3/54·8**	48/20/38/56	**0·38/2·75/1·5/0·0**	8/1/6/7	25/100/50/0
240518	36·3/32·8/23·5/19·0	33/34/4/2	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
244121	23·7/17·8/23·8/24·0	10/9/6/6	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
244175	26·8/3·8/26·0/28·5	17/1/10/16	0·0/1·0/0·0/0·0	9/6/14/7	0/25/0/0
248181	51·8/39·3/**48·3/58·8**	52/48/44/63	0·0/0·0/0·75/0·0	9/11/10/7	0/0/25/0
249647	25·8/29·5/23·5/23·0	15/28/4/4	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
250440	52·8/50·0/52·5/43·8	54/63/52/36	0·0/0·0/**0·0/0·5**	9/11/14/5	0/0/0/25
250442	37·8/36·0/33·0/37·5	35/40/22/28	0·0/0·0/0·63/0·0	9/11/11/7	0/0/25/0
261623*	34·0/42·0/**64·0/45·7**	29/54/64/40	0·0/0·0/**0·0/1·17**	9/11/14/3	0/0/0/67
261631	30·5/19·0/27·3/32·5	23/11/12/22	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
263029	23·5/26·8/41·0/41·8	9/21/36/34	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
263031*	23·0/19·25/34·5/28·0	8/12/27/15	0/0/**1·0/0**	9/11/8/7	0/0/75/0
263032	37·8/41·8/**56·8/42·8**	35/53/57/35	0·5/0·63/0·0/0·0	7/9/14/7	25/25/0/0
269770	**39·3/17·8**/32·5/26·3	38/9/19/11	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
269778	13·7/16·8/**26·8/36·3**	2/8/11/27	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
269793	38·0/38·5/55·0/58·3	36/46/55/62	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
269802	28·0/17·8/21·5/23·0	20/9/3/4	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
270536**	41·5/28·5/**38·8/48·5**	40/23/32/46	**2·13/0·0**/1·13/0·0	2/11/7/7	75/0/50/0
271510	24·8/22·8/35·0/30·5	13/18/29/18	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
272171	48·8/31·3/64·8/57·8	48/29/66/60	0·0/0·0/**0·0/0·75**	9/11/14/5	0/0/0/25
273209	46·3/46·5/·/61·0	44/59/·/67	0·0/0·0/·/0·0	9/11/·/7	0/0/·/0
275822	20·0/20·5/33·3/27·3	4/15/23/13	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
279825	31·5/34·3/34·0/25·0	24/36/25/9	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
280236*	48·8/50·5/64·3/67·5	48/64/65/70	0·0/0·0/**3·13/0·0**	9/11/1/7	0/0/100/0
280611*	30·3/33·0/**34·3/56·7**	22/35/26/59	0·5/0·63/**2·25/0·0**	7/9/2/7	50/25/75/0
280613	29·8/22·3/28·0/24·8	21/16/13/8	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
280626	24·5/18·7/**18·7/31·3**	12/10/2/20	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
285719	**59·3/74·8/51·8/71·3**	64/73/50/74	0·0/0·0/0·25/0·0	9/11/13/7	0/0/25/0
285720	63·3/78·8/62·8/69·8	65/74/63/72	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
285724	25·0/14·3/28·5/25·0	14/5/14/9	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
285739	47·8/47·0/50·3/55·8	46/60/48/58	0·0/0·0/0·83/0·0	9/11/9/7	0/0/33/0
285747	**63·5/39·0/50·0/60·5**	67/47/47/65	0·0/0·0/0·25/0·0	9/11/13/7	0/0/25/0
286430*	34·3/36·5/46·3/44·3	30/41/42/37	0·0/0·0/1·0/0·0	9/11/8/7	0/0/50/0
324693	31·8/31·7/**45·8/33·5**	25/30/41/25	0·0/0·0/0·5/0·0	9/11/12/7	0/0/25/0
324695	40·8/40·7/45·0/50·3	39/52/40/49	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
343972	54·7/51·7/·/47·5	59/65/·/44	0·0/0·0/·/0·0	9/11/·/7	0/0/·/0
343975	·[h]/46·3/41·7	·/·/43/33	0·0/0·0/0·0/0·0	9/11/14/7	0/0/0/0
343977*	43·0/44·5/·/60·0	41/58/·/64	0·0/1·25/·/0·38	9/5/·/5	0/50/·/25
343988	44·25/34·67/**60·5/45·25**	43/37/61/38	**0/0/0·63/1·0**	9/11/11/4	0/0/25/50

Table 2 *Continued*

Genotype[a]	Lesion length[b]	Lesion rank[c]	Nodal resistance[d]	Nodal rank[e]	Percentage survival[f]
344000	·/72/·/58·0	·/72/·/61	0/0/·/0	9/11/·/7	0/0/·/0
344003	49·0/39·25/**43·75/60·75**	49/48/39/66	0/0·625/0·63/0	9/9/11/7	0/25/25/0
344538**	33·0/33/·/62·25	27/35/·/68	**2·13/1**/·/0	2/6/·/7	100/100/·/0
347281*	**43·0/20·25**/51·0/54·25	41/14/49/55	1·5/0/**0/0·25**	4/11/14/5	50/0/0/25
347501	**37·5/50/28·57/53·25**	34/63/15/53	0/0/0·75/0	9/11/10/7	0/0/25/0
358300	**68·25/40·25/56·0/70·5**	68/51/56/73	0/0/0/0	9/11/14/7	0/0/0/0
371796	**33·0/15·25**/37·0/35·0	27/7/31/26	0/0·88/0/0	9/7/14/7	0/50/0/0
378157	35·3/32/46·25/46·75	32/32/42/42	**0/0·63**/0/0	9/9/14/7	0/25/0/0
378160	59·25/61·5/56·75/55·25	64/69/57/57	0/0/0/0	9/11/14/7	0/0/0/0
381334	23·75/14/**24·5/32·75**	11/4/7/23	0/0/0/0	9/11/14/7	0/0/0/0
391630	**46·75/23·5/40·25/51·25**	45/19/34/51	**0·75/0**/0·25/0	6/11/13/7	25/0/25/0
404220	49·0/62/**45·75/61·0**	49/70/41/67	0/0/0/0	9/11/14/7	0/0/0/0
411141	20·25/10·3324·67/22·25	5/3/8/3	0/0/0/0	9/11/14/7	0/0/0/0
411142	34·75/24·75/32·0/28·5	31/20/18/16/	0/0/0/0	9/11/14/7	0/0/0/0
413683	33·67/28·67/30·67/31·0	28/25/16/19	0/0/0/0	9/11/14/7	0/0/0/0
413688	38·33/37·33/30·67/31·0	37/43/16/19	0/0/0/0	9/11/14/7	0/0/0/0
413695	**20·75/32·0**/32·75/32·	6/32/20/21	0/0/0/0	9/11/14/7	0/0/0/0
413697*	26·25/28·0/43·75/51·75	16/22/39/52	**1/0**/0·25/0	5/11/13/7	50/0/25/0
413698	30·25/22·5/14·75/28·0	22/17/1/15	0/0/0/0	9/11/14/7	0/0/0/0
419217	**21·25/37·5/48·5/72·0**	7/44/45/75	**1/0**/0/0	5/11/14/7	25/0/0/0
429853	56·0/60/**72·0/35·0**	62/68/70/26	0/0/0/0	9/11/14/7	0/0/0/0
499982	44·0/40/58·25/49·33	42/50/59/48	0/0/0/0	9/11/14/7	0/0/0/0
505112	**24·75/47·25/59·25/40·5**	13/61/60/32	0/0/0/0	9/11/14/7	0/0/0/0
560058	63·33/50/·/67·67	66/63/·/71	0/0/·/0	9/11/·/7	0/0/·/0
560071*	52·25/65·67/·/57·75	53/71/·/60	**2·88/0**/·/0	1/11/·/7	100/0/·/0
639980	**68·5/38·25**/57·5/45·5	69/45/58/39	0/0·88/0/0	9/7/14/7	0/25/0/0
639981	56·5/53·75/64·0/51·25	63/67/64/51	0/0/0/0	9/11/14/7	0/0/0/0
1203–1	14·5/·/·/36·25	3/·/·/27	0/0/·/0	9/·/·/7	0/0/·/0
1204–2	24·75/23·5/23·67/9·0	13/19/5/1	0/0/0/0	9/11/14/7	0/0/0/0
1204–3	11·25/9·67/25·67/33·0	1/2/9/24	0/0/0/0	9/11/14/7	0/0/0/0
LSD[i]	14·85/13·60/12·63/10·87	NA[j]/NA	0·66/0·79/0·90/0·55	NA/NA	NA/NA

[a]Number of asterisks next to genotype numbers indicate the number of trials in which the genotype had a nodal resistance of 1 or greater and a percentage survival of 50% or greater among the four trials.
[b]Mean lesion length in mm after 72 h in a humidity chamber.
[c]Ranks the mean lesion lengths of each genotype from the least to greatest.
[d]Mean nodal resistance based on a 0 (highly susceptible) to 4 (highly resistant) scale (see Materials and methods for details.).
[e]Ranks the mean nodal resistance of genotypes from the greatest to the least.
[f]Percentage of plants surviving 2 weeks post-inoculation.
[g]Data for trials 1 to 4 shown as: Trial 1 data/Trial 2 data/Trial 3 data/Trial 4 data; data that in bold indicates a significant difference in the ranking of a specific pea genotype for lesion length or nodal resistance when comparing trials 1 and 2 or 3 and 4, respectively.
[h]Indicates missing data.
[i]Least significant difference among genotypes within a trial according to Fisher's LSD.
[j]NA = not applicable.

Future research will develop mapping populations from these resistant accessions to identify genes associated with the observed resistance.

Previous research noted that pea genotypes with quantitative partial resistance to *S. sclerotiorum* tended to have thick stem diameters (Blanchette & Auld, 1978). Correlation coefficients comparing mean lesion length and stem thickness in the present study were assessed to verify this hypothesis. The present study determined that the correlation between stem diameter and lesion length had a significant ($P = 0{\cdot}003$) negative correlation indicating that accessions with large stem diameters tended to be more resistant than accessions with smaller stem diameters based on mean lesion length. Therefore, stem diameter is an important resistant factor that needs to be incorporated into breeding lines for resistance to *S. sclerotiorum*. Future research will address whether increasing stem diameter is correlated with factors that determine the strength of the stem, such as lignin content, which may inhibit lesion movement, or if genotypes with thicker stems tend to be more vigorous and tolerate invasion more than thin-stemmed genotypes.

Many pea genotypes that restricted lesion advancement did not survive 2 weeks post-inoculation. Only 4, 7, 6 and 4 of the top 30 genotypes with the shortest lesion lengths, survived in trials 1 and 2 of both greenhouse and laboratory

Table 3 Pearson correlation coefficients and *P*-values for correlations between internode length, stem diameter and stem crushing strength versus stem shearing strength, lesion length, nodal resistance and survival of 62 pea accessions from the *Pisum* core collection when plants were inoculated with *Sclerotinia sclerotiorum* and maintained at 10°C in the laboratory (Trials 1 and 2) or 15 to 28°C in a greenhouse (Trials 3 and 4) in a humidity chamber for 3 days

	Trial 1			Trial 2		
Variable[a]	Lesion length	Nodal resistance	Survival	Lesion length	Nodal resistance	Survival
Internode	0·214 (0·097)	0·101 (0·441)	0·115 (0·377)	0·236 (0·064)	0·120 (0·352)	0·218 (0·088)
Diameter	–0·517 (< 0·0001)	–0·210 (0·105)	–0·271 (0·035)	–0·352 (0·005)	–0·237 (0·063)	–0·184 (0·153)
Crushing	–0·151 (0·254)	0·100 (0·451)	0·062 (0·638)	–0·074 (0·574)	–0·159 (0·225)	–0·151 (0·250)
Shearing	–0·311 (0·015)	–0·094 (0·471)	–0·127 (0·328)	–0·146 (0·257)	–0·145 (0·260)	–0·101 (0·437)
	Trial 3			Trial 4		
Internode	0·281 (0·027)	0·069 (0·592)	0·134 (0·301)	0·134 (0·299)	0·063 (0·6241)	0·107 (0·4081)
Diameter	–0·376 (0·003)	–0·113 (0·381)	–0·055 (0·671)	–0·276 (0·030)	–0·144 (0·264)	–0·133 (0·304)
Crushing	–0·218 (0·094)	0·143 (0·276)	0·168 (0·200)	–0·032 (0·806)	–0·020 (0·882)	–0·004 (0·974)
Shearing	–0·180 (0·161)	–0·003 (0·979)	0·080 (0·534)	–0·175 (0·174)	0·046 (0·723)	0·086 (0·508)

[a]Information regarding internode length, stem diameter, crushing strength and stem shearing strength were obtained and downloaded from GRIN.

Table 4 Pea accession number, lesion length, nodal resistance, survival, seed colour, plant height, flower colour, species, leaf type and origin of the 22 most resistant accessions to *Sclerotinia sclerotiorum* in the *Pisum* core collection[a]

Genotype	Lesion length[b] (mm)	Nodal resistance[c]	Survival[d]	Seed colour[e]	Flower colour	Species	Origin
103709	17·0	3·125	1	green/white	white	*P. sativum*	India
156720	21·75	0	0	green/white	white	*P. sativum*	Japan
164972	48·0	.[f]	> 0	mixed	pigmented	*P. sativum*	Turkey
165949	56·5	.	> 0	green/white	pigmented	*P. sativum*	India
166084	19·75	0	0	green/white	pigmented	*P. sativum*	India
169603	24·75	.	> 0	pigmented	white	*P. sativum*	Turkey
171810	18·33	0	0	green/white	pigmented	*P. sativum*	Turkey
197044	20·75	3·25	1	green/white	pigmented	*P. sativum*	Honduras
227258	45·0	0	0	green/white	pigmented	*P. sativum*	Iran
240515	18	.	0·60	green/white	white	*P. sativum*	India
261623	33·25	0	0	green/white	pigmented	*P. sativum*	Spain
263031	9·5	1·25	0·5	green/white	white	*P. sativum*	France
270536	21·25	2	0·5	green/white	pigmented	*P. sativum*	Denmark
280236	56·0	2·75	0·5	green/white	pigmented	*P. sativum*	Ethiopia
280611	37·5	3·75	1	green/white	white	*P. sativum*	Ukraine
286430	41·75	0·875	0·25	green/white	pigmented	*P. sativum*	Nepal
347281	29·75	1·75	0·5	green/white	white	*P. sativum*	India
413697	22·0	2·875	1	green/white	white	*P. sativum*	Hungary
343988	43·5	3·5	1	green/white	pigmented	*P. s. sativum*	Turkey
343977	55·5	1·25	0·25	green/white	white	*P. s. elatius*	Turkey
344538	79·33	1·25	0·5	green/white	.	*P. s. elatius*	Italy
560071	46·0	3	0·25	pigmented	white	*P. s. pumilio*	Israel

[a]Lesion length and nodal resistance values were taken from the primary screening trials for these genotypes.
[b]Length of lesion after 3 days in a humidity chamber.
[c]Mean nodal resistance based on a 0 (highly susceptible) to 4 (highly resistant) scale (see Materials and methods for details.).
[d]Percentage of plants surviving 2 weeks post-inoculation.
[e]Data for origin, seed colour, flower colour and origin were obtained from GRIN.
[f]Indicates missing data.

screenings, respectively (Table 2). This indicates that although these genotypes ranked the highest in restricting lesion advancement, the lesions continued to expand in the majority of these genotypes until the plant was killed. Therefore, white mould resistant genotypes need to combine lesion restriction with nodal or internodal resistance that inhibits lesion expansion down the stem. It has been observed that in resistant bean and pea stems inoculated with the white mould pathogen that as the lesion reaches a node, the advancement of the lesion is slowed or completely inhibited. Advancing lesions on plants with short internodes would encounter nodes more frequently than

plants with longer internodes, which may explain why internode length and lesion size were significantly negatively correlated in two of four greenhouse and laboratory trials.

Nineteen of the 22 genotypes identified with quantitative partial resistance are described in the Germplasm Resources Information Network (GRIN) established by the United States Department of Agriculture, Agricultural Research Service (http://www.ars-grin.gov/npgs/pullman.html) as having non-pigmented (green/white) seed and ten of these genotypes are also white-flowered (Table 4). Many of the pea accessions in the core collection have coloured flowers and pigmented seed, but the pea industry is currently focused in developing cultivars with non-pigmented seed and white-flowers due to the horticultural characteristics and industry demands associated with these traits. The present study identified 10 non-pigmented/white flowered genotypes that can be used in breeding for resistance to *S. sclerotiorum* that would facilitate the development of white mould-resistant cultivars with desired non-pigmented seed and white flowers (Table 4).

None of the pea genotypes with quantitative partial resistance to *S. sclerotiorum* were of the afila (semi-leafless) leaf type. The afila leaf type is currently desired by pea food processors because it allows for greater penetration of sunlight into the plant canopy resulting in a more uniform pea colour at harvest. In addition, the afila type reduces canopy foliage, which reduces the development of high humidity in the canopy that tends to favour white mould development (Kraft & Pfleger, 2001). Future breeding efforts for white mould resistance should incorporate the quantitative partial resistance from the pea genotypes identified in this study with the afila leaf type to form pea cultivars with genetic and architectural resistance to the white mould pathogen.

Previous research assessing resistance to white mould in peas used oat grains colonized with *S. sclerotiorum* to screen pea genotypes (Blanchette & Auld, 1978). The colonized oat was placed on the soil, touching the base of the stem. This technique was previously tested in the current programme to screen pea genotypes for white mould resistance. However, preliminary tests determined that non-colonized oats placed next to the pea stem of several peas in replicated trials promoted the development of a water-soaked lesion, even when using pasteurized soil. Potentially, compounds released from sterilized oats that come in contact with the stem promote stem tissue to break down. Hence, the technique was replaced with the currently described mini-agar plug technique, which never resulted in any false positives.

Research using the colonized oat inoculation technique identified 39 PI accessions from the *Pisum* collection with quantitative partial resistance to white mould (Blanchette & Auld, 1978). Only six (263027, 261622, 197990, 272175, 269778 and 210583) of these 39 PI accessions were represented in the core collection that was presently screened, and none of these accessions were identified as being resistant to *S. sclerotiorum* under the screening conditions. Differences in isolates of *S. sclerotiorum* used in the two studies may account for the differences, or resistance may vary depending on the stem inoculation site. The study using the colonized oat technique tested for white mould resistance at the interface between the above and below ground stem, whilst the present laboratory study tested the resistance of the upper stem. Both inoculation sites, at the base of the stem and at the fourth node, represent potential natural infection sites. However, both inoculation sites may provide different information regarding quantitative partial resistance of these genotypes. Additional research needs to assess the resistance of genotypes to multiple isolates of *S. sclerotiorum* and difference in quantitative partial resistance based on stem inoculation location.

In the western USA, isolates of *S. sclerotiorum* collected from California and Washington, and Ontario, Canada were characterized for genetic diversity using a DNA fingerprinting technique (Malvárez *et al*., 2007). The *S. sclerotiorum* isolates from California and Ontario were collected from lettuce, and the Washington isolates from peas and lentils. Populations from all three locations were determined to represent three genetically differentiated populations. The California population had the most diverse population, while the populations from Ontario and Washington were clonal. Additional populations of *S. sclerotiorum* taken from potatoes were determined to be highly diverse (Atallah *et al*., 2004). Since populations of *S. sclerotiorum* have been found to be both clonal and highly diverse, it would be important to screen the white-mould resistant pea genotypes identified in the present study with additional *S. sclerotiorum* isolates from other pea growing regions within Washington and North America to identify the robustness of the resistance identified in the present research.

The private pea lines characterized as 'woody stem lines' were screened for white mould resistance because the stems are considered to have high lignin concentrations. High lignin content in the stems theoretically has the potential to prevent or reduce infection and lesion movement by *S. sclerotiorum*. The woody stem lines tended to have shorter lesion lengths than the mean lesion length of other *Pisum sativum* lines across the 11 screening sessions, $4{\cdot}65 \pm 1{\cdot}89$ to $2{\cdot}51 \pm 1{\cdot}01$, respectively. However, six of the seven lines did not survive 2 weeks post-inoculation in the primary screening tests. Line 1204-3 restricted lesion length to the greatest level of all woody lines screened, but did not survive 2 weeks post-inoculation in repeated greenhouse and laboratory tests (Table 2), indicating that woody-stem types retard lesion advancement but the lesions continue to advance.

The high percentage of genotypes that did not survive 2 weeks post-inoculation and the lack of genotypes showing high levels of quantitative partial resistance (lesion lengths less than 1·0 cm) to *S. sclerotiorum*, illustrated the aggressiveness of this pathogen to peas and the limited breeding material available to breeders. Resistance to *S. sclerotiorum* in peas is not likely associated with a single gene, since the mean lesion length data from the 11 primary

screenings was not bimodal (Fig. 1), which would indicate that white mould resistance in peas is likely associated with quantitative genetic resistance.

Based on the current research, recommendations to pea breeders developing white-mould-resistant peas would be to make crosses with pea genotypes identified in the present study that had both quantitative partial resistance to lesion expansion and nodal or internodal resistance to prevent the continued advancement of the lesion. Characteristics such as a thick stem diameter, short internode and the afila leaf type should also be incorporated to develop highly effective and commercially acceptable white mould-resistant cultivars.

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